Gentiana kurroo Royle – A critically endangered bitter herb

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Abstract: Gentiana kurroo Royle is a critically endangered bitter drug plant of western and northwestern Himalaya. The secondary plant metabolites present in the root and rhizome of this plant are valued as bitter tonic, antiperiodic, expectorant, antibilious, astringent, stomachic, anthelmintic, blood purifier, carminative, antipsychotic, anti-inflammatory, antibacterial and sedative. It is also medicated for curing skin diseases, leucoderma, leprosy, bronchial asthma, dyspepsia, flatulence, colic, anorexia, helminthiosis, inflammations, amenorrhea, dysmenorrheal, strangury, hemorrhoids, constipation and urinary infections. The drug plant is heavily extracted for root and rhizome. Because of restricted distribution and widespread extraction from its natural habitats coupled with nil cultivation, this species is in high risk category as far as its survival is concerned. The paper presents a comprehensive review on its morphophenology, ethnopharmacy, regeneration techniques and phytochemistry in a lieu to assist further research in chemical screening for elite genotype, developing agrotechniques of cultivation, varietal development and byproduct extraction and thereby protecting the miracle plant from extinction.

Keywords: Gentiana kurroo; Morphophenology; Ethnopharmacy; Phytochemistry; Endangered bitter plant.

Introduction

Gentianaceae is a cosmopolitan family of flowering plants comprising of 87 genera and 1615-1688 species. The members of this family are annual and perennial herbs or shrubs & are native to northern temperate areas of the world except Antarctica. The genus Gentiana contains about 360 species (Struve and Albert 2002; Daniel and Sabnis 1978), of which 62 species are observed in India (Sunita and Bhattacharyya 1982). Plants belonging to genus Gentiana are very popular for the bitter glycosides they contain and have been widely used in traditional medicine for treatment of different human disorders.

Gentiana kurroo Royle a member of this family is a critically endangered medicinal herb of the western and northwestern Himalayan biomes. Although not mentioned by Sanskrit writers in Materia Medica (Dymock 1890), it is a significant drug of ayurveda (Shahi 1993). Vernacularly it is known as Karu in Hindi, Traayamaana in Sanskrit and Indian gentian in English (Jain 1968; Satyavati et al. 1976; Khare 2007). The dried roots and rhizomes are official in Indian pharmaceutical codex. The bitter glycosides (gentiopicrin & gentianin), alkaloids (gentiomarin) etc. present in the root posses immense medicinal and pharmaceutical importance. The root stock is valued as bitter tonic, antiperiodic, expectorant, antibilious, astringent, stomachic, anthelmintic, blood purifier and carminative (Kirtikar and Basu 1935). It is also used for curing skin diseases, bronchial asthma and urinary infections (Chopra et al. 1956; Anonymous 1956). In trade it is often substituted for true gentian obtained from Gentiana lutea, a native of Europe and Asia Minor (Dutt 1928). Because of its high medicinal value, the plant species has been over exploited from its natural habitat and no efforts are underway for replenishment and skilled harvesting. Efforts towards domestication have not been successful so far due to poor seedling establishment (Raina et al. 2011). The paper presents a comprehensive review on its morphophenology, ethnopharmacy, propagation
techniques and phytochemistry in a lieu to assist further research in chemical screening of elite genotype, developing agrotechniques of cultivation, varietal development and byproduct extraction and thereby protecting the miracle plant from extinction.

**Morphology**

*Gentiana kurroo* is a perennial herb (Figure 1). The aerial part is mostly composed of radical leaves which occur in a rosette. The shoot is represented by flowering branches only which bears cauline leaves. Root stock (rhizome and adventitious root) is white to brown in colour, surface is vertically wrinkled and terminates in a scaly tuft consisting of bases of leaves and flowering shoot. Radical leaves are long, narrow, simple, sessile, stipulate, lanceolate, lamina entire, apex acute, leathery in texture and basally joined in pairs forming a common sheath. Cauline leaves are narrow linear and in pairs united at base forming a tube around flowering shoot. Inflorescence is monochasial cymose type. Flowers are large in size, infundibuliform, bracteates, pedicellate, complete, hermaphrodite, actinomorphic, hypogynous, pentamerous and deep violet blue from outside with corolla white from inside below plicae. Sepals are five, gamosepalous, persistent, tubular, lobes linear, more or less equal in size. Odd sepal is posterior to mother axis. Petals five, gamopetalous, infundibuliform, five lobed limbs and the tips are obtuse. The odd petal is anterior to mother axis. Sepals and petals are persistent up to fruit maturation stage. Stamens are five, free, epipetalous and alternating with petal lobes. Ovary is bicarpillar, syncarpous, unilocular and superior with parietal placentation. Ovule are numerous and anatropous. Style is indistinguishable. Fruit is a capsule and dehisces longitudinally. The species is a genomic allotetraploid with n=13 (Behera and Raina 2011b).

**Phenology**

Shoot of this perennial herb is represented by flowering branches only. Stem is a modified rhizome. Two types of leaves i.e. radical leaves at the base of the plant and cauline leaves on flowering shoot are present. The radical leaves are deep green in colour and remain throughout the life cycle of the plant and gradually replaced by newer leaves. Most of the new leaves are developed upon onset of rainy season. The cauline leaves are initially green in colour and gradually fade its colour with maturity of inflorescences. They shed along with the flowering shoots. Flowering occurs during Sept-October with 1-9 inflorescences per plant. Large funnel shaped protrandrous flowers are entomophilous and cross pollinated. Different insect vectors seen visiting flowers of *Gentiana kurroo* are bumble bee (*Bombus* spp.), honey bee (*Apis melifera*), lady bird beetle (*Coccinella septempunctata*). Capsule takes 18-20 days to mature after fertilization. The first fort night of November is the ideal time for seed harvest. Seeds are light in weight and 1000 seed weighs 0.1275 gm (Behera and Raina 2011b; Raina et al. 2003).

**Ecology**

It is endemic to Northwestern Himalayas and commonly grows in Kashmir, Himachal Pradesh and adjoining hills of northwestern Himalaya between 1500-3000 m m.s.l (Chaudhary and Wadhwa 1984). In Himachal Pradesh it has become sporadic in subalpine to alpine meadows. It can be collected from Kalga patten (4200 m a.s.l.) , Dainasor rocky slopes (4500-5000 m a.s.l.), Pathrala Thatch, Gajyani catchment (4000 m a.s.l.), Dhamkhari marvi, Karal tibba in Solan and Mangarh area of Sirmour district at lower altitudes at 2000m (Chauhan 1999). Plants of this species grow luxuriantly on southern open exposed slopes of mid and higher mountain ridges. Such sites are characterized by low temperature, high insolation, heavy wind speed and surrounded by tall grasses & scrubs and prefers to be overshadowed by them. *Themeda anathera*, *Artemisia absinthium*, *A. scoparia*, *Asparagus filicinus*, *Bidens cernua*, *Erigeron multicaulis*, *Hackelia macrophylla*, *Indigofera heterantha*, *Lespedeza elegans*, *Medicago minima*, *Rosa macrophylla*, *Sium latijugum*, etc. are the companion species associated with Karu. Among these *Themeda anathera* is the dominant species (Khuroo et al. 2005).
Status of the plant

The drug plant is heavily extracted for root and rhizome which are official in Indian pharmaceutical codex. Because of restricted distribution and widespread extraction from its natural habitats coupled with nil cultivation, this species is in high risk category as far as its survival is concerned. The red data book of Indian plants list this as endangered and its status is critical (Tandon 1997). According to IUCN this species is declared endangered and its cultivation is not known (Molur and Walker 1998). Hence government of India has put this species in the negative list of export vide notification no. 2(RE-98)1997-2002 dated 13 April 1998.

Figure 1: Gentiana Kurroo, A. Vegetative stage, B. Flowering stage.

Pharmacognostical characters of crude Drug

Adulteration either intentional or unintentional reduces potency of the drug. High price coupled with extreme scarcity of raw material leads to intentional adulteration. Gentiana kurroo is known to be substituted/adulterated with the roots of Picrorrhiza kurroa, Gentiana tenebrosa, G. decumbens, Exacum bicolor etc. (Datta and Mukerji 1949; Sreelatha et al. 2007). Macroscopic and anatomical observations along with chemical analysis are some features used for judging the purity and authenticity of crude drugs (Trease 1949).

Macroscopical character

A peculiar characteristic of this species is the splitting of the older rhizome into four parts. After secondary growth, the rhizome splits anomalously into four parts at a distance of 2.02 ± 0.435 cm near collar region. These four parts seem to fuse into a single structure which is triangular or quadrangular in outline. Rhizome surface is vertically wrinkled and terminate in a scaly tuft consisting of bases of leaves and flowering shoots.

Microscopical character

Transverse section of leaf and root reveals that leaf is dorsiventral, stomata anamocytic, and present only on the lower surface. Stomatal index ranges from 24.26 - 25.78 and palisade ratio is 5.68. Xylem elements in roots are polycyclic and radialy arranged.

Physical constants

The air dried rhizome after incerneation gives 4.06 per cent of total ash (cremish colour),
2.78 per cent of acid soluble ash and 8.03 per cent of sulphated ash. Calcium content is 0.28 per cent. These macro & microscopic characters along with physical parameters can be used as identification tool for testing the purity of raw material of *G kurroo* (Behera and Raina 2011a).

### Propagation

*Gentiana kurroo* can be propagated through seeds, rhizome cuttings, micro proliferation of shoot nodal segments and somatic embryogenesis.

#### I). Propagation through Seed

Flowering occurs during September to October and capsule takes 18-20 day to mature after fertilization. First fortnight of November is the ideal time for seed harvest. Seeds should be stored at low temperature (below 5°C) after harvesting otherwise there is considerable reduction in germination percentage. Seeds more than one-year-old lose viability and do not germinate. June is the ideal time for seed sowing and 70-75% seeds germinate (Raina et al. 2003). The commencement of germination starts sixth day onwards after sowing and continued up to 28 days (Tomar 2011). Although the plants set numerous seed & seed germinates abundantly, the seedling establishment is very poor (Raina et al. 2011). Premature anther development is the reason behind this (Badola & Pal 2002). Seedling should be potted as soon as first two leaves are formed, without disturbing the root system (Bailey 1929). Karu requires a well drained stony gravelly soil rich in humus for its better growth (Tomar et al. 2011).

#### II). Propagation through rhizome cuttings

Macro proliferation through rhizome cuttings is an effective method of propagation of *Gentiana kurroo*. Rhizomes of 5-8 cm diameter are longitudinally split into two pieces in such a way that each piece should contain above ground part of mother rhizome with growing buds. Cuttings are treated with rooting hormones (IAA or IBA) and planted in raised bed. Among the rooting hormones IBA is more preferred and gives better result (Tomar et al. 2011).

#### III). Micropropagation

**a). Clonal propagation through shoot proliferation**

Rapid clonal multiplication through auxiliary branching is a simple procedure of propagation of *Gentiana kurroo*. Shoot tips and nodal segments of mature plants are washed in teepol detergent (about 15 min.) followed by cleaning in running tap water (2 hrs). Subsequently they were surface disinfected with 0.1% HgCl₂ for 10 min and rinsed with sterile distilled water before implanting vertically on nutrient medium. MS (Murashige & Skoog) media supplemented with 6% sucrose (pH should be at 5.8) is the most suitable nutrient medium for shoot proliferation and about 90-100% rooting achieved (Sharma et al. 1993). The clonal plantlets thus produced are genetically stable and in vitro propagation of this species can be done on commercial basis indefinitely without any risk of genetic instability (Kaur et al. 2009).

**b). Somatic embryogenesis**

Seedlings, organs of regenerants, callus tissue cell suspension and isolated protoplasts of *Gentian kurroo* bears high potential for somatic embryo formation and shoot regeneration (Fiuk et al. 2003). Protoplasts can be derived from any type of plant cell but embryogenic suspension with a high morphogenic potential is the best source (Fiuk & Rybczynski 2007). Amongst root, hypocotyls and cotyledon in seedling explants, cotyledon derived cell suspension bears the highest morphogenic potential in MS agar medium supplemented with 0.5-1.0 mg*l⁻¹* Kin, 0.5mg*l⁻¹* GA₃, and AS mg*l⁻¹*80 (Fiuk & Rybczynski 2008). The efficiency & success of regeneration of *Gentiana kurroo* by means of protoplast culture is affected by the source of cell suspension culture, enzyme treatment, size of aggregate fraction, composition of culture medium (Fiuk & Rybczynski 2007). The photosynthetic activity of the somatic embryo derived germilings would be most efficient when the
culture medium is supplemented 0.2-0.4% sucrose (Ryczynski et al. 2007).

Ethnopharmacy

The root stock of G. kurroo is valued for presence of the most known bitter compounds like gentianine & amaroswerin. Gentianine possesses anti-inflammatory, analgesic, anticonvulsant, hypotensive, antipsychotic, sedative, diuretic, antimalarial, antiamoebic and antibacterial properties and amaroswerin gastroprotective (Singh 2008). In Indian system of medicine the root stock is valued as bitter tonic, antiperiodic, expectorant, antibilious, astringent, stomachic, anthelmintic, blood purifier and carminative, antipsychotic, anti-inflammatory, sedative, anti-bacterial (Anonymous 1956 & Kirtikar & Basu 1935). It is also medicated for curing skin diseases, leucoderma, leprosy, bronchial asthma, dyspepsia, flatulence, colic, anorexia, helminthiosis, inflammations, amenorrhea, dysmenorrheal, strangury, hemorrhoids, constipation and urinary infections (Chopra et al. 1956 and Warrier et al. 1995). It is an important ingredient of many tonics for stomachic preparation (Pulliaiah 2002). Because of its similar properties the root stock is many times substituted for Gentiana lutea, the true or European gentian (Dutt 1928 and Dey & Bahadur 1973).

In folk lore medication Neilkanthi leaf powder mixed with oil is applied on ulcer and fungal infection (Unial & Shiva 2005). The root is used in stomachache and is used in urinary infections (Gilani et al. 2006). Decoction is made from the root with ginger root powder is used for curing high fevers. Tea made from the root and cinnamon bark is used as a tonic. A powder made from the root, dried lemon peel, and cardamom fruit (Elettaria cardamomum) is taken with a locally brewed beer called "chang" as a valuable tonic (Sharma 2000). Amchi (traditional doctors) of Lahul & Spiti use whole plant against fever, cough, headache, liver ailments and as blood purifier (Sharma et al. 2006). The drug is very helpful in removing all kinds of debility and exhaustion of body from prolonged illness, improves digestive system and lack of appetite. The root is used as an ingredient in preparing feed for fattening horses (Qureshi et al. 2007). In unani system of medicine the flower tops (Gule-Ghafis) has been traditionally used for treatment of inflammation, pain, fever and hepatitis (Latif et al. 2006). Amongst these ethnomedical properties anti-inflammatory activity of flower tops (Latif et al. 2006) and analgesic activity of roots (methanolic extracts) of Gentiana kurroo (Wani et al. 2011a) is scientifically validated.

Phytochemistry

The rhizome & roots of this herb contains some of the most known bitter glycosides like Gentiopicrine, Gentianamarin, Gentianine (alkaloid), Amaroswerin (Anonymous 1956 and Singh 2008), 6-cinnamoyl catapol, 6-O-feruloyl catapol, 6-O-vanillyl catapol, 6-O-cinnamoyl catapol, aucubin, catapol (Sarg et al. 1991 and Rodriguez et al. 1998), Genianic acid, pectin, uncrystallizable sugar (Nadkarni 1976), tannins, saponins, cardiac glycosides, terpenes, flavonoids, phenolics, and carbohydrates (Wani et al. 2011a).

Leaves also contain some bitter compounds significantly higher than roots (Raina et al. 2011). It contains iridoid glycoside 2’-(2, 3-dihydroxybenzoyloxy)-7-ketologanin (Kumar et al. 2003) and 16 volatile aroma compounds (Wani et al. 2011b). Among the volatile aroma compounds dimethyl sulphide, 2-ethylfuran, 1,8-cineole, α-terpinyl acetate, methandriol are the major compounds. Other chemical compounds like 1, 3-propanediol, 2-methyl sulphide, 3-methyl butanol, pentanol, hexanal, 7-oxabicyclo (4,1,0)-heptanes are present in minor quantities (Wani et al. 2011b). Flower tops contain alkaloids, flavonoids, glycosides, free phenols and terpenes (Latif et al. 2006).

Conclusion

Though the drug plant is in high demand in traditional Ayurvedic medicine formulation and pharmaceutical industry no commercial cultivation is observed in any part of the country. This is may be due to the unavailability of proper package of practice of cultivation of Gentiana kurroo. Most of the works have been concentrated on morphophenology, cytology, breeding,
Review on Indian bitter herb Gentiana kurroo regeneration, phytochemistry and ethnobotanical study. In spite of a wide range of medicinal utility, only anti-inflammatory and analgesic activity of crude drug is being scientifically validated. Therefore there is a huge scope for pharmacological study. A comprehensive strategy is necessary for phytochemical screening for elite genotype, varietal development, development of agrotechniques of cultivation and standardization of protocol for byproduct extraction. Domestication of this wild drug plant will not only reduce pressure from natural population, it will save the plant from extinction.

References


